replaced with chicken D137L Tm showed a 0.15 increase in pCa_{50} compared to WT. This result supports the hypothesis (Sumida et al., 2008) that the increased flexibility imparted to the Tm coiled-coiled structure by Asp at 137 avoids excessive turning-on of the system at the high physiological myosin concentrations. Supported by Telethon-Italy GGP07133, STREP Project ''BIG-HEART'' 241577 EC and NIH HL22461.

1164-Plat

Skeletal Muscle Myopathy Mutations at the Actin-Tropomyosin Interface that Cause Gain Or Loss of Function

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Two mutations, skeletal α-actin K326N and β-tropomyosin ΔK7 were reported in patients with 'stiff' muscles and spontaneous contractures, suggesting a hypercontractile phenotype. K326N actin was isolated from a patient biopsy and ΔK7 β-tropomyosin was expressed in baculovirus/sf9 cells. Ca^{2+} regulation of reconstituted thin filaments was studied by quantitative *in vitro* motility assay. Both mutations increased Ca^{2+}-sensitivity (EC_{50} mut/WT $=0.37 \pm 0.05$ and 0.45 ± 0.25 respectively). In recent models of actin-α-tropomyosin in the OFF state, both actin K326 and tropomyosin K7 are located in the actin-tropomyosin interface. We suppose that the charge change due to the mutation would destabilise the OFF state and favour the equilibrium towards the ON state, thus accounting for the higher Ca^{2+}-sensitivity.

We also examined a pair of mutations that cause loss of function. Skeletal α-actin D292V (from a patient biopsy) and β-tropomyosin E117K (expressed in baculovirus/sf9 cells) cause congenital fibre type disproportion (i.e weak contractility without nemaline bodies). In reconstituted thin filaments β-tropomyosin E117K caused a decrease in Ca^{2+}-sensitivity (EC_{50} mut/WT $= 2.44 \pm 0.55$). Addition of tropomyosin to actin D292V filaments caused a complete switch-OFF of motility that could not be reversed by troponin at high Ca^{2+} or even NEM-S-1. Thus congenitally weak muscles correlate with loss of function at the molecular level. These mutations are not at the interface of the OFF state but have an opposite charge change to the gain-of-function mutations and are in a location that could destabilise the ON state, which may account for the loss of function.

1165-Plat

Testing Predictions of a Simple Two-State Model of Thin Filament Regulation: Inhibitors that can Activate Thin Filament Motility

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Striated muscle is activated by calcium binding and further activated when myosin heads strongly bind the regulated thin filament (RTF). Several intermediate states of the myosin-RTF binding pathway continue to be debated, however for an accurate mesoscopic kinetic model it is imperative to define only stable chemical states without inclusion of transient intermediate structures. Our novel simplified model of thin filament regulation (SMoR) is a streamlined, two-state system with calcium- and myosin-dependent binding rates. This minimalist model is consistent with both the Hill and the McKilloop and Geeves models. It is easily incorporated into larger scale models of skeletal and cardiac mechanics and provides a simple analytical expression with powerful predictive value for accurate hypothesis testing. SMoR predicts amrinone, an inhibitor of ADP release, will increase calcium sensitivity; sucrose, an inhibitor of myosin attachment, will decrease calcium sensitivity as a result of modulations of attachment and detachment kinetics, respectively. Our experimental data shows pCa50 shifts from a standard value of 5.65 to 6.44 with 4mM amrinone; and to 5.14 with 180mM sucrose. Our model predicts that altering both attachment and detachment kinetics will have compensatory effects, allowing calcium sensitivity to be ''rescued''. Complex modulations of myosin kinetics were tested experimentally with 4mM amrinone which was rescued with low myosin density and 120mM sucrose to restore the pCa50 to 5.64 and 5.66 respectively. SMoR predicts that decreasing the ADP release rate at low calcium can increase actin-sliding velocities. Consistent with the prediction, we show amrinone increase actin-sliding velocities at sub-activating calcium concentrations. The ability to make complex predictions from fundamental principles has yet to be shown by any other model. SMoR is an accessible and accurate alternative to more complex models of regulation.

Platform: Membrane Protein Structure I

1166-Plat

Testing the Structural Mechanism of Transmembrane Translocation using De-Novo Designed Soft-Metal Transporters with Inverted Dual Topology

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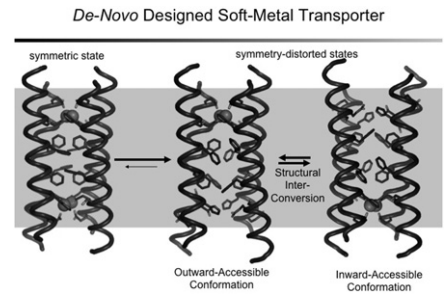
Recent crystallographic discoveries show that surprisingly diverse transmembrane transporters share a very similar topology composed of pseudo-symmetric inverted dual subunits. So, a common structural mechanism may govern the divers translocation. For instance, alternate frustration of the pseudo-symmetry can thought to be the underlying principle that governs the structural interconversion that results in alternate access of substrates. Here, to test the generation of transport function by a universal mechanism, we have employed a de-novo design approach to achieve a soft metal translocation function in a minimalist system. An anti-parallel homo-tetrameric bundle of 25-residue transmembrane helices was designed to adopt soft metal chelation in a pseudo inverted symmetry context. Vesicle-reconstituted de-novo peptide shows transport activity specific for soft metal ion Zn2+. Also, the protein structure and chelation, as well as its tetrameric arrangement, gazed by UV-Vis, circular dichroism, analytical ultracentrifugation, NMR and x-ray diffraction in a micellar context is consistent with the design. Together, these results are in agreement with transport via alternate frustration of internal symmetry.



1167-Plat

Transporter BetP - Oligomeric Structure, Transport Catalysis and Regulation

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The Na+ coupled betaine uptake system BetP of Corynebacterium glutamicum belongs to the BCCT family of transporters and comprises both a catalytic function (betaine/Na+ cotransport) and a sensory/regulatory function (responding to osmotic stress). Its 2D (electron crystallography) and 3D structure (X-ray crystallography) has been solved. Within a homooligomeric trimer, each BetP protomer harbours both an N- and a C-terminal domain involved in stimulus sensing and intramolecular signal transduction. Factors known so far contributing to the sensory and regulatory function of BetP are (i) the two terminal domains, (ii) K+ ions as an osmostress related stimulus, and (iii) interaction with the surrounding membrane. The primary stimulus of BetP, the rise in the cytoplasmic K+ concentration, has been elucidated using a proteoliposomal system, whereas the second stimulus, attributed to changes in the physical state of the surrounding membrane was investigated in intact cells.

Intramolecular signal transduction of the two different stimuli involves contributions from individual domains of BetP protomers and is essentially based on its oligomeric (homotrimeric) structure. We have now analyzed interaction of the three individual protomers of BetP in mechanistic terms by using novel heterooligomeric constructs of BetP composed of three structurally different protomers. On the basis of these studies we suggest a functional model of intersubunit crosstalk between the three individual BetP monomers as well as the terminal domains of BetP during its catalytic and its sensory function.

1168-Plat

Three-Dimensional Structure of Full Length Integrin Embedded in Membrane

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Electron cryo-microscopy, three-dimensional image analysis and computational docking were used along with biochemical and biophysical approaches to provide the three-dimensional (3D) structure of full length $\alpha_{IIb}\beta_3$ integrin while in a membrane bilayer. Integrins are expressed on the cell surface in either an ''on'' or an ''off'' state with respect to ligand binding. Increased